Remarks

The Interview

The undersigned greatly appreciates the opportunity to interview this case with the examiner and his supervisor. This response is being filed prior to the interview since the supervisor is not available until after the six month non-extendible deadline to file a response to the office action.

Amendments to the Claims

The claims have been significantly narrowed to define three specific bacterial strains for which evidence of unexpected results was provided in the application: *Ralstonia eutropha, Pseudomonas putida* and *Escherichia coli* producing polyhydroxyalkanoate.

These amendments are made solely to facilitate prosecution and should not be construed as an admission that applicants are not entitled to the substantially broader claims that have been pending and are being pursued in a continuation application.

Withdrawn claims 12, 14-16 and 21 have been amended to refer to the "process" of claim 11, from which they depend directly or indirectly.

Claim Objections

Claims 1 and 7 were objected to for containing typographical errors. Claims 1, 7 and withdrawn claims 11 and 19 have been amended to correct the spelling of "lysed".

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-18, 13 and 18 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to

reasonably convey to one skilled in the art that the inventor had possession of the claimed invention.

Claims 1-8, 13 and 18 were also rejected under 35 U.S. C. § 112, first paragraph as allegedly containing new matter. Claims 1, 7, and withdrawn claims 11 and 19 have been amended to remove reference to lysis by osmotic shock, rendering this rejection moot. Support for release of nuclease upon lysis of the claimed bacteria can be found original claim 8.

From the description in the specification and knowledge in the art (discussed below), one of ordinary skill in the art would conclude that Applicants were in possession of the claimed bacterial strains.

The claims define a bacteria strain for production of polyhydroxyalkanoates, genetically modified to express a heterologous nuclease gene, which is secreted into the periplasmic space and released when the cells are lysed. Claim 1 and the claims dependent therefrom specify that the bacteria is selected from the group consisting of *Ralstonia eutropha, Pseudomonas putida* and *Escherichia coli.*

The nuclease genes needed to make the claimed bacteria are known in the art as acknowledged by the Examiner (office action, page 4). Furthermore, suitable nuclease genes were well known and described in the literature with specific sources taught in the specification at least at page 6, lines 4-13, and can be obtained and produced by using well established methods in the art, such as PCR and primers complementary to the sequence encoding the nuclease using information obtained from publicly available databases. Examples of such sequences are disclosed for many strains in GenBank (see at least page 6, lines 4-13; and page 7,

lines 15-22). Once the nuclease gene has been isolated, common genetic manipulation allows for its integration into a microbial strain (see at least page 7, lines 8-10). Thus, the nuclease genes are adequately described by provision of Genbank accession numbers.

In *Falkner*, the Federal Circuit recently addressed the issue of written description in an appeal from an interference. *Falkner v. Inglis*, 448 F.3d 1357, 79 U.S.P.Q.2d 1001 (Fed. Cir. 2006) the Federal circuit clarified that there is no per se rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure. *Falkner* at 1366. Furthermore, the Board of Patent Appeals and Interferences noted that the written description requirement does not require a description of the complete structure of every species within a chemical genus. (*see Utter v. Hiraga*, 845 F.2d 993, 998, 6 U.S.P.Q.2d 1709, 1714 (Fed. Cir. 1988), stating "A specification may, within the meaning of 35 U.S.C. § 112, para. 1, contain a written description of a broadly claimed invention without describing all species that claim encompasses". Therefore, Applicants submit that one of ordinary skill in the art would conclude that Applicants were in possession of the claimed bacterial strain.

However, the Examiner stated that while the genes and methods of making the claimed mutants, as well as methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis etc, are well known, producing variants as claimed by Applicants (*i.e.*, bacterial strain comprising any heterologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid) requires that one or ordinary skill in the art know or be provided with guidance for the selection of which of the infinite number of variants have the claimed property. Applicants respectfully draw the Examiner's attention to the

claims as amended. The claims do not recite "mutants"; the claims do not recite "modification". The Examiner's allegations appear to be verbatim to the allegations made in the office action mailed on December 7, 2006, relevant to the claims pending at that time, which recited "mutants" and "modification". This is also true of the Examiner's allegation of a lack of disclosure of any particular structure to function relationship with respect to those genetic modifications of homologous nuclease (office action, page 6); the claims do not recite "homologous". The Examiner's allegation that the claims are drawn to any heterologous nuclease gene as well as any genetic modification thereof (office action, page 7) is also incorrect.

The claims were amended on October 24, 2007 (with the filing of an RCE) to define: a bacterial strain for production of a fermentation product selected from the group consisting of antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates , and polysaccharides, wherein the bacterial strain is genetically modified to express a heterologous nuclease gene wherein the nuclease gene product is secreted into the periplasmic space and released when the bacteria is lysed by osmotic shock. The claims are currently amended to recite specific bacteria that produce polyhydroxyalkanoates and remove reference to lysis by osmotic shock. For at least the reasons stated above, the claims satisfy the written description requirement.

With respect to the Examiner's requirement that the specification provide guidance for the selection of which of the infinite number of variants have the claimed property, and that without such guidance one of ordinary skill in the art would be reduced to the necessity of producing and testing all of the virtually infinite possibilities, this is not the standard for written

description. The standard is that the specification describe that which is claimed in such a way as to reasonably convey to one of ordinary skill in the art that the inventor had possession of the claimed invention.

The Examiner has failed to provide a reason why the examples of nuclease genes described in the specification (with gene bank accession numbers) does not satisfy the requirement for a representative number of species.

The examiner has failed to provide any evidence or reasoning as to why those skilled in the art would not use the examples in the application as guidance in using other strains of bacteria or other nuclease genes (see the specification at least at page 6, lines 9-10) and screen for strains expressing desired levels of nuclease as Applicants have done for heterologous expression of the *Staphylococcus aureous* nucleas in *P. Putida*.

The Examiner's allegation of a lack of written description appears to be directed to limitations not recited in the claims, based on undue experimentation, which is not the legal standard.

Applicants submit that for at least the reasons set forth above, the claims meet the written description requirement.

Rejection Under 35 U.S.C. § 102

Claims 1-8 were rejected under 35 U.S.C. § 102(b) as anticipated by Liebl, *et al.*, *J. Bacteriology* 174(6):1854-1861 (1992) ("Liebl"). Claim 1 has been amended to specify that the bacteria is selected from the group consisting of *Ralstonia eutropha, Pseudomonas putida* and

Escherichia coli. Applicants respectfully traverse this rejection as applied to the amended claims.

The Legal Standard

For a rejection of claims to be properly founded under 35 U.S.C. § 102, it must be established that a prior art reference discloses each and every element of the claims. *Hybritech Inc. v. Monoclonal Antibodies Inc.*, 231 U.S.P.Q. 81 (Fed. Cir. 1986); *Scripps Clinic & Research Found. v. Genentech Inc.*, 18 U.S.P.Q.2d 1001 (Fed. Cir. 1991). The Federal Circuit held in *Scripps*, 18 U.S.P.Q.2d at 1010:

> Invalidity for anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention.

A reference that fails to disclose even one limitation will not be found to anticipate, even if the missing limitation could be discoverable through further experimentation. As the Federal Circuit held in *Scripps*:

> [A] finding of anticipation requires that all aspects of the claimed invention were already described in a single reference: a finding that is not supportable if it is necessary to prove facts beyond those disclosed in the reference in order to meet the claim limitations. The role of extrinsic evidence is to educate the decision-maker to what the reference meant to

persons of ordinary skill in the field of the invention, not to fill in the gaps in the reference.

Id.

For a prior art reference to anticipate a claim, it must enable a person skilled in the art to make and use the invention. "A claimed invention cannot be anticipated by a prior art reference if the allegedly anticipatory disclosures cited as prior art are not enabled". *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1354, 65 U.S.P.Q.2d 1385, 1416 (Fed. Cir. 2003).

Analysis

Liebl does not recite all of the claim limitations.

Liebl does not disclose the claimed bacterial strain selected from the group consisting of *Ralstonia eutropha, Pseudomonas putida* and *Escherichia coli*, genetically modified to express a nuclease gene which is secreted into the periplasmic space. Liebl discloses Staphylococcal nuclease (SNase) expression by various *C. glutamicum* strains, wherein the *C. glutamicum* transgenic strain is used for investigating protein export and processing. The nuclease in Liebl is secreted into the culture medium. Applicants respectfully submit that as of the time of publication of Liebl, it was widely believed that gram positive bacteria did not have a periplasmic space (see Sakamoto, *et al.*, *Microbiology*, 147:2865-2871 (2001), submitted by Applicants with the amendment and response filed on October 24, 2007, which specifically states "gram positive bacteria have no outer membrane or periplasmic space".) Thus, although experiments have subsequently shown (in Zuber, *J. Bacteriol.*, 188(18):6652-60 (2006); ("Zuber"; cited by the Examiner) that gram positive bacteria do have what is considered a

periplasmic space, Liebl was not aware of this in 1992, let alone what genes/proteins were involved in gram positive bacteria; thus Liebl could not enable one of skill in the art to genetically engineer the *C. glutamicum* disclosed in Liebl to secrete nuclease into a space that he, by the common knowledge in the art, did not believe existed (assuming that *C. glutamicum* does indeed have such a space). Furthermore, an engineered protein is not invariably secreted into the periplasmic space of bacteria. One must engineer the protein providing the necessary sequences for such secretion into the periplasmic space, as opposed to extracellular secretion (*i.e.*, across the cell membrane and into culture medium). Liebl does not enable one of skill in the art to modify *C. glutamicum* for secretion of nuclease into a periplasmic space, let alone the bacteria recited in claim 1.

With respect to claim 7, Liebl does not disclose an integrated gene, but a plasmid that requires induction for expression.

Thus, Liebl does not disclose all of the claim limitations as required for a rejection under 35 U.S.C. §102(b) and cannot anticipate the claims.

Therefore, claims 1-8 are not anticipated by Liebl.

Rejection Under 35 U.S.C. § 103

Claims 1-10 were rejected under 35 U.S.C. § 103(a) as obvious over WO 94/10289 by Greer, *et al.*, ("Greer"), Atkinson, *et al.*, Biochemical Engineering and Biotechnology Handbook, 2nd Edition, Stockton Press: New York, 1991 ("Atkinson") and Lee, *et al.*, *Adv. Biochem. Eng. Biotechnol.* 52:27-58 (1995) ("Lee"), or Miller, *et al.*, *J. Bacteriology* 169(8):3508-3514 (1987) ("Miller") in view of Liebl or Miller. Applicants respectfully traverse this rejection.

The Legal Standard

When applying 35 U.S.C. § 103, the following tenets of patent law must be adhered to:

(a) determining the scope and contents of the prior art;

(b) ascertaining the differences between the prior art and the claims in issue;

(c) resolving the level of ordinary skill in the pertinent art; and

(d) evaluating evidence of secondary consideration.

Graham v. John Deere, 383 U.S. 1, 17-18, 148 U.S.P.Q. 459, 467 (1966). These four factors are traditionally referred to as the Graham factors.

Obviousness is a legal conclusion. *See Graham v. John Deere Co.*, 383 U.S. 1, 17-18, 148 U.S.P.Q. 459 (1966). The *Graham* analysis was recently affirmed by the Supreme Court in *KSR Int'l Co. v. Teleflex, Inc.*, 127 S. Ct. 1727, 82 U.S.P.Q.2d 1385 (2007).

The obviousness analysis requires looking at the invention as a whole. "Focusing on the obviousness of substitutions and differences, instead of on the invention as a whole, is a legally improper way to simplify the often difficult determination of obviousness." *Gillette Co. v. S.C. Johnson & Sons, Inc.*, 919 F.2d 720, 724, 16 U.S.P.Q.2d 1923 (Fed. Cir. 1990); *see Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1383, 231 U.S.P.Q. 81, 93 (Fed. Cir. 1986).

Hindsight analysis, such as picking and choosing from prior art references using the claimed invention as a template, has long been forbidden. *See, e.g., In re Fine*, 837 F.2d 1071, 1075 (Fed. Cir. 1988), which states that "One cannot use hindsight reconstruction to pick and choose among isolated disclosures on the prior art to deprecate the claimed invention." In *KSR*, the Court also warned against the use of hindsight analysis in making an obviousness

determination. The Court stated, "A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning." (*KSR*, 127 S. Ct. at 1742, citing *Graham*, 383 U.S. at 36 (warning against a "temptation to read into the prior art the teachings of the invention in issue" and instructing courts to "guard against slipping into the use of hindsight" (quoting M*onroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F.2d 406, 412, 141 U.S.P.Q. 549 (6th Cir. 1964))).

Analysis

The Scope and Content of the Prior art

Greer

Greer describes the exogenous addition of peroxide to a cell culture. As stated in the Examples of Greer, and as stated as one of the problems addressed by the presently claimed invention, the exogenous addition of nucleases is generally known and too expensive to use for commodity fermentation products involving high cell density fermentations.

Liebl

Liebl describes the heterologous expression of a *Staphylococcus aureus* nuclease gene in *C. glutamicum* and the use of this transgenic system for investigating protein export in *C. glutamicum*, as discussed above.

Miller

Miller discloses the use of a *B. subtilis* secreted nuclease for investigating "the nature of the processing of the nuclease signal peptide". Miller further characterizes the secretion of nuclease and the processing of the signal peptide from the precursor protein in *B. subtilis*. Miller

speculates that the *staphylococcal* nuclease and its gene may be very useful for the development of secretion vectors for foreign proteins.

Atkinson

Atkinson is a general review of biochemical and biotechnological methods and reagents.

Lee

Lee reports on production of PHAs in bacteria, and control of fermentation conditions.

The Differences Between the Prior Art and the claims

A combination of Greer, Liebl, Miller, Atkinson and Lee does not recite all of the elements of the claims.

The claims define a bacterial strain selected from the bacteria recited in claim 1, for production of polyhydroxyalkanoates, wherein the bacterial strain is genetically modified to express a heterologous nuclease gene wherein the nuclease gene product is secreted into the periplasmic space and released when the bacteria is lysed. Liebl and Miller disclose genetically engineering the gram positive bacteria *C. glutamicum* and *B. subtilis* respectively, to secreted nuclease into the culture medium. As noted above in response to the 102 (b) rejection, this is not tantamount to a disclosure of secretion of nuclease into the periplasmic space as claimed. The claimed bacterial strains are engineered to (1) produce large amounts of nuclease which is (2) secreted into the periplasm where it is harmless to the cell, until release by cell lysis. None of Greer, Atkinson or Lee makes up for these deficiencies. Greer is not concerned with genetically engineering bacterial strains to secrete nuclease. Lee discloses the production of copolyesters in

Pseudomonas sp. Lee does not disclose genetically modifying any bacteria for secretion of protein into the periplasmic space. Atkinson, a review of biochemical and biotechnological methods and reagents, similarly does not make up for this deficiency. The availability of biotechnology tools does not make obvious results obtained from their use. Biotechnology tools have been around for a long time; however, it is still not possible to obtain expression of genes in certain organisms or expression to desired levels; see for example Makrides, *et al.*, *Microbiol. Rev.*, 60(3):512-538 (1996) ("Makrides", a copy of which is attached), which states for example, "in spite of the extensive knowledge on the genetics and molecular biology of *E. coli*, not every gene can be expressed efficiently in this system" (see page 512, right col.). One of ordinary skill in the art is aware that the availability of biotechnology tools does not make obvious their application absent specific instructions on how to successfully apply the tool for the intended purpose.

With respect to claim 7, none of the prior art discloses genetically modifying bacteria with the heterologous nuclease gene integrated into the chromosome, and the gene product secreted into the periplasmic space.

Evidence of secondary considerations

As the Court reiterated in *KSR v. Teleflex*, evidence of long standing need and of commercial success are both secondary indicia of non-obviousness. Secondary considerations to be considered include commercial success, long felt but unresolved needs, failure of others, unexpected results, etc.

Microbrial fermentations are used for the manufacture of a large number of products. Increased productivity and recovery of more highly purified product are major areas of development to increase profitability. Decreasing fermentation costs is another means for increasing profitability.

When bacterial cells are grown at high cell densities (a requirement in large scale fermentation processes) and lysed, the cells produce a highly viscous jelly-like mass due to nucleic acids released from cells following lysis. Solutions such as exogenous nuclease addition, hydrogen peroxide or heat treatment are undesirable because exogenous nuclease is expensive, and hydrogen peroxide or heat treatment can negatively impact product quality. Furthermore, mixing an external nuclease/hydrogen peroxide into this mass to break down the nucleic acids can be very difficult due to high viscosity. The densities to which cell cultures can be grown are therefore limited by the strength of the pumps used to mix the external nuclease into the lysed cell mixture and the cost of the nuclease. One is therefore given a choice between growing cells at lower densities or having difficulty getting the product out of the cell mass.

The claimed method is based on the discovery by Applicants, of a way to endogenously produce nuclease and direct its secretion into the periplasmic space of bacteria. The cells are engineered to (1) produce large amounts of nuclease which is (2) secreted into the periplasm where it is harmless to the cell. When the cells are lysed, the nuclease is released and begins breaking down the nucleic acids. External mixing is not essential because the nuclease is already mixed into the cell mass. The claimed process allows fermentations at high cell densities, because with endogenous production of nuclease which is released upon cell lysis, the

fermentation process is no longer dependent on exogenous nuclease, and is therefore not limited by pump strength (required to mix in the exogenous nuclease).

With respect to claim 7, chromosomal integration of the heterologous nuclease and expression of nuclease to high levels (shown in Example 6) avoids the use of plasmids which are difficult and expensive to maintain in large scale fermentations, as well as the use of IPTG which is cost prohibitive and toxic (see Makrides, page 514, left col.) that has been necessary in plasmid-based expression systems in the prior art, in order to obtain appreciable expression/secretion of nuclease. Such cost effective strains are highly desirable. For example, medium chain length polyhydrodroxyalkanoate (MCL-PHA) polymers are not on the market yet; these polymers have to compete with materials such as polyutherenes whose cost of production varies between 2-5 $/kg (see review by Weusthuis, *et al.*, in BIOPOLYMERS, eds. Steinbuchel, *et al.*, pp 291-317, WILEY-VCH Verlag GMBH, Rep of Germany, 2002, a copy of which is attached; see especially page 311). Thus, there is still a need for methods, bacterial strains, or methods of fermentation which enable avoidance of some of the costs associated with large scale fermentations. The claims provide microbial strains which are cost effective for fermentation processes and can enable more profitable production of the products listed in claim 1.

Accordingly, claims 1-8 are not obvious over Greer, Aktinson, and Lee or Miller in view of Liebl or Miller.

Allowance of claims 1-4, 6-8, 11, 12, 14-16, 19 and 21 is respectfully solicited. Claims 11, 12, 14-16, 19 and 21 are related to claims 1-8 as product and process of use. Accordingly, no new search would be required should claims 1-4 and 6-8 be found to be allowable.

Respectfully submitted,

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Date: July 24, 2008

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